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Effect of side chain location in (2-aminoethyl)-aminomethyl-2-phenylquinolines as antitumor agents

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Abstract

Three new derivatives of 2-phenylquinoline having an (2-aminoethyl)aminomethyl group in 7-, 6-, or 4'-(para position of 2-phenyl ring) positions of aromatic system have been prepared. The antitumor activity of these compounds together with 8- or 4- substituted isomers against the HeLa cell is in the order of 8- > 7- > 4- ≈ 6- ≈ 4'- substituted ones, which is almost in good agreement with that of DNA-binding ability evaluated by means of DNA-titration of UV-VIS spectra, DNA melting experiment, and ethidium displacement assay. Two representative compounds (8- and 4- isomers) are confirmed to have an ability to intercalate into double stranded DNA by topoisomerase I superhelix unwinding assay.

Keywords: Acridines; Antitumour compds; DNA; Polycyclic heterocyclic compds

DNA attractive molecules having an appropriate binding power are considered as effective drugs for cancer chemotherapy of solid tumors since these molecules might be able to spread to broad sphere of tumor tissues.[1] Denny *et al.* demonstrated the efficacy of 2+1 unfused tricyclic aromatic systems such as phenylquinolines and benzimidazoles as 'minimal intercalators' that have moderate binding affinity with DNA.[2] Therefore, it is an exciting trial to introduce the metal-coordinating group to these fascinating aromatic systems in the developments of metal containing drugs. Ethylenediamine group is one of the most simple, convenient, and popular ligands, that has already been introduced to acridine,[3] distamycin in the form of Schiff base[4] and the other DNA-binding molecules.[5] This type of diamine is also applicable to syntheses of Schiff base, nylon, and the other useful macromolecules. Thus, among a wide variety of application on an ethylenediamine ligand, platinum(II) complexes have been extensively demonstrated to have a significant potential as a related drug of cisplatin.[3,5] In the course of this study, we have already prepared the cisplatin-type platinum(II) complexes

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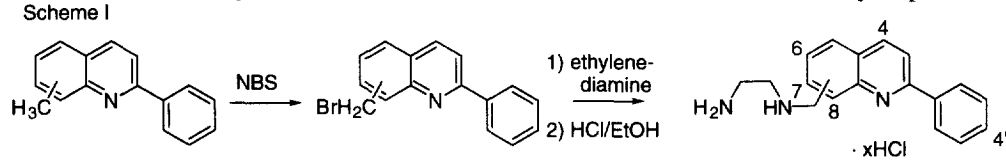
linked to 4- and 8-positions of 2-phenylquinolines as a candidate of modurated drug of cisplatin.[6] Here, we wish to report the systematic study of synthesis of ethylenediamine ligands linked to 2-phenylquinoline in different positions and the evaluation of their antitumor activity and DNA-binding capacity.

1. Synthesis: Synthesis of compounds **1** and **4** were previously reported.[6] The methyl groups of 6-, 7-, and 4'(para position of 2-phenyl ring)-methyl-2-phenylquinoline were converted to bromomethyl group by NBS in the presence of AIBN or BPO (23-80 %) and followed by reaction with ethylenediamine in anhydrous acetonitrile or acetone to give corresponding (2-aminoethyl)aminomethyl-2-phenylquinolines **1-5** (11-51 %) (Scheme I). All the compounds except **2** were obtained in a form of trihydrochloric salt.[7] It should be noted that compound **2** which was obtained as a dihydrochloric salt did not dissolve into water sufficiently at pH = 6.

Scheme I



1: 4-, x = 3
2: 6-, x = 2
3: 7-, x = 3
4: 8-, x = 3
5: 4'-, x = 3

2. UV-VIS titration by calf thymus DNA: The electronic absorption spectrum of aqueous buffer solution of compound **4** changed as shown in Fig. 1.

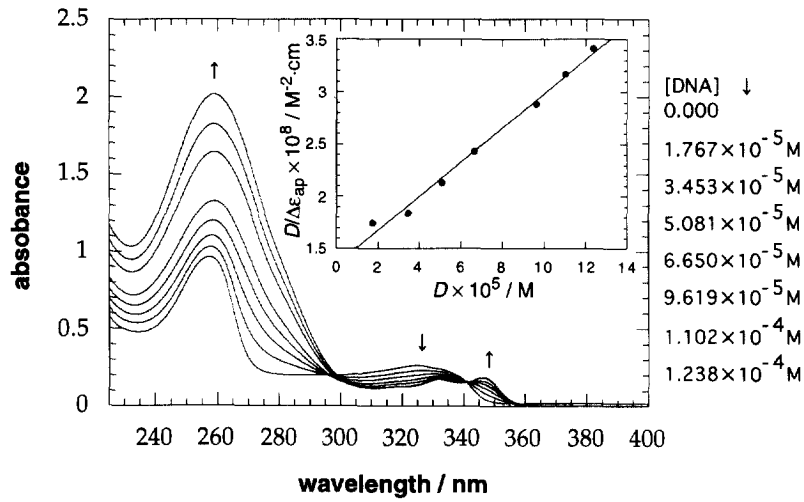


Figure 1. Absorbance change of compound **4** (2.5×10^{-5} M) in the presence of increasing amount of calf thymus DNA in 1.0 mM sodium cacodylate buffer containing 4.0 mM NaCl (pH 6.0) at 25 °C. Inset: Plot of $D/\Delta\varepsilon_{ap}$ *vs* D at 325 nm. See text.

The red-shift and hypochromism around 300-360 nm region, which can be explained by typical intercalation, and isosbestic points at 296 (prevented by absorbance of DNA at later stage of titration) and 341 nm were observed. From the spectral change at 325 nm, we estimated the binding constant (K_{app}) with calf thymus DNA at 9.7×10^3 M^{-1} according to eq 1,[8]

$$D/\Delta\varepsilon_{ap} = D/\Delta\varepsilon + 1/(\Delta\varepsilon \times K_{app}) \quad \text{(eq 1)}$$

where, D is the DNA concentration, $\Delta\varepsilon_{ap} = |\varepsilon_a - \varepsilon_f|$, and $\Delta\varepsilon = |\varepsilon_b - \varepsilon_f|$; $\varepsilon_a = A_{obs}/[drug]$, ε_f and ε_b are molar extinction coefficients of free and DNA-bound drug, respectively. The plot of $D/\Delta E_{ap}$ *vs* D was presented in inset of Fig. 1.

Unfortunately, we could not obtain the K_{app} of the other compounds because they exhibited no clear isosbestic points on the similar titration conditions, indicating the existence of another binding mode(s).

3. DNA melting: The thermal DNA denaturation experiments were applied to investigate the DNA-binding capacity of the ethylenediamine 2-phenylquinoline conjugates. Fig. 2 shows the enhanced thermal stability of the duplex in the presence of 50 % (in bp) of ligand molecules. ΔT_m values at [drug]/[DNA base pair] ratio (r) of 0.1, 0.3, and 0.5 are summarized in Table 1. The extent of duplex stabilization depends significantly on the nature of an added ligand. Compound **4** stabilizes DNA duplex most strongly among all the drugs examined in the present study. It is surprising that compound **1** has less stabilizing effect of duplex than **4** even in the 5-fold concentration (comparing the ΔT_m values of **4** at $r = 0.1$ with that of **1** at $r = 0.5$) in spite of that they have the same functional groups.

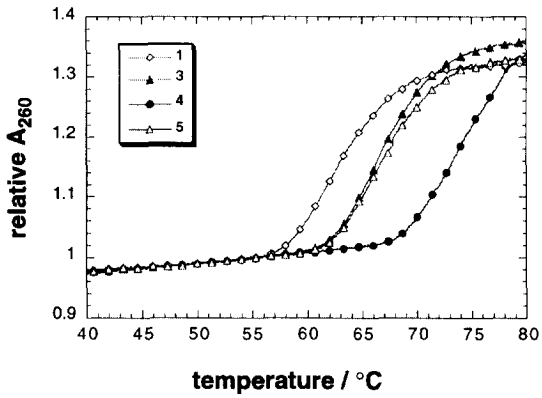


Figure 2. UV-melting curves of calf thymus DNA in the presence of 50 % (in bp) of 2-phenylquinoline derivatives.

Table 1. Difference in Melting Temperature (ΔT_m) of Calf Thymus DNA in the Presence of 2-Phenylquinoline Derivatives[a]

	ΔT_m (°C)		
Compound	$r = 0.1$	$r = 0.3$	$r = 0.5$
1	3.5	6.5	7.6
3	6.0	11.0	13.3
4	8.7	14.2	17.7
5	5.6	8.8	11.5

[a] r = [drug]/[DNA].

4. Ethidium displacement: The fluorescent ethidium bromide displacement assay was also examined to evaluate the DNA-binding affinity of these drugs. C_{50} values (the micromolar drug concentrations necessary to reduce the fluorescence of initially DNA-bound ethidium by 50 %) of compounds were obtained using 0.5 μM (in bp) of DNA (1.0 mM sodium cacodylate buffer containing 4.0 mM NaCl (pH 6.0)) with 1.26 μM of ethidium at 25 °C.[9] The results are summarized in Table 2.

Table 2. C_{50} Values[a] in the Ethidium Displacement Assay Bound to Calf Thymus DNA, Poly[d(AT)]$_2$, and Poly[d(GC)]$_2$[b]

Compound	C_{50} (μM) Calf Thymus	Poly[d(AT)]$_2$	Poly[d(GC)]$_2$	$\frac{C_{50}(GC)}{C_{50}(AT)}$
1	30	33	48	1.5
3	11	11	16	1.5
4	2.1	5.2	5.6	1.1
5	7.0	7.0	11	1.6

[a] See text.
[b] [DNA] = 0.5 μM, [ethidium] = 1.26 μM.

The order of strength of binding to DNA was **4** > **5** > **3** > **1**, which is almost coincident to that obtained from DNA denaturation experiment (**4** > **3** ≥ **5** > **1**). Compound **4** binds to DNA about ten times larger than compound **1**. It should be noted that apparent AT-specificity is observed in compounds **1**, **3**, and **5**, which do not exhibit any distinct isosbestic points in UV-VIS DNA titration experiment, *i.e.* these compounds might have a capacity of minor groove binding in addition to the intercalative binding mode.

5. Supercoiled DNA unwinding assay: To confirm the intercalation ability of these compounds, plasmid DNA (pBR322) was treated with topoisomerase I in the presence of compounds **1** and **4**. The mixture of pBR322 (30 μM in bp), topoisomerase I (12.5 unit), and drug (0-480 μM) in 10 mM Tris-HCl (pH = 8.0) containing 72 mM of KCl and 5 mM of $MgCl_2$ was incubated for 2 hours at 37 °C. The relaxed plasmid DNA was analyzed by 1 % agarose gel electrophoresis after removal of enzyme and drug by phenol extraction. Fig. 3 shows the enhanced superhelix unwinding by increasing amount of drugs, where partially relaxed ladder in lanes 6-8 strongly support the intercalation of these drugs. Here again, the compound **4** was found to intercalate to DNA double helix more strongly than **1**.

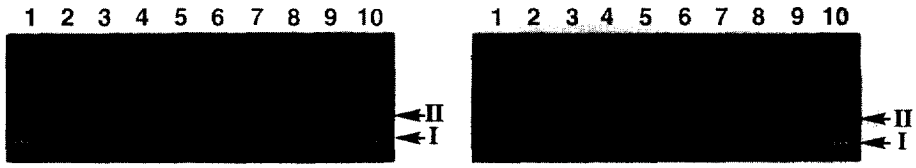


Figure 3. Superhelix unwinding of pBR322 DNA by topoisomerase I in the presence of 2-phenylquinoline derivatives **1** (left) and **4** (right). [pBR322] = 30 μM/bp, [topoisomerase] = 12.5 unit. Lanes 1 and 10 are intact DNA, lane 2 is topoisomerase control; (left) lanes 3-9: [**1**] = 15, 30, 60, 120, 240, 360, 480 μM; (right) lanes 3-9: [**4**] = 3, 15, 30, 45, 60, 90, 120 μM.

6. Cytotoxicity: The cytotoxicity of **1-5** were assayed against the Human uterine cancer cell (HeLa cell) line. 10^3 cancer cell incubated in the growth media (200 μl of MEM contains 10 % of Fetus Bovine Serum) at 37 °C overnight was grown in the presence of drugs for more 72 hours and number of survive cell was analyzed by MTT assay. The acridine derivatives corresponding to 2-phenylquinoline compound **1**, 9-(2-aminoethyl)aminomethylacridine trihydrochloride (**6**)[6] were also examined. Results are summarized in Table 3. The trend of cytotoxicity of the compounds (**4** > **3** > **1** ≈ **2** ≈ **5**) is almost in good agreement with that of DNA-binding ability. The cytotoxicity of platinum complexes of ligands **1** and **4** also corresponds to present results.[10]

The effect of 2+1 unfused tricyclic aromatic systems of 2-phenylquinoline ring was small toward the HeLa cell line in the present experimental conditions rather than the effect of side chain location. The binding constant of this acridine derivative **6** (3.4-3.6 × 10^4 M^{-1})[6] might be reasonable to explain this small difference.

Table 3. Cytotoxicity of 2-Phenylquinoline Derivatives against the HeLa Cell Line[a]

Drug Conc. (M)	Cell Survival (%) 1	2	3	4	5	6
1.0×10^{-7}	74±7	85±7	98±8	77±17	79± 8	~100
1.0×10^{-6}	62±3	82±2	70±1	65±22	87±11	94±10
1.0×10^{-5}	52±4	44±1	31±2	7± 2	65± 9	71± 3
1.0×10^{-4}	8±1	~0	3±1	~0	3± 2	3± 2

[a]Errors are in standard deviation.

In summary, the location of ethylenediamine side chain in 2-phenylquinoline derivatives have a large effect to control the DNA binding ability, DNA binding mode, and cytotoxicity of the compounds. These results are useful in exploring the diamine-functionalized drug of 2-phenylquinolines.

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9. The extent of fluorescence quenching due to non-displacement mechanism were estimated to be small by the similar methods employing excess amount (20 μM) of DNA and 2 μM of ethidium.
10. HeLa cell survival (%) and drug concentration (M)[6] for **1**·$PtCl_2$: 99 at 1.0×10^{-7}, 74 at 1.0×10^{-6}, 70 at 1.0×10^{-5}, ~0 at 1.0×10^{-4}. For **4**·$PtCl_2$: 100 at 1.0×10^{-7}, 71 at 1.0×10^{-6},15 at 1.0×10^{-5}, 2 at 1.0×10^{-4}.